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# MHC presentation via autophagy and how viruses escape from it

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**Abstract**

T cells detect infected and transformed cells via antigen presentation by major histocompatibility complex (MHC) molecules on the cell surface. For T cell stimulation these MHC molecules present fragments of proteins that are expressed or taken up by the cell. These fragments are generated by distinct proteolytic mechanisms for presentation on MHC class I molecules to cytotoxic $CD8^+$ and on MHC class II molecules to helper $CD4^+$ T cells. Proteasomes are primarily involved in MHC class I ligand, and lysosomes in MHC class II ligand generation. Autophagy delivers cytoplasmic material to lysosomes, and, therefore, contributes to cytoplasmic antigen presentation by MHC class II molecules. In addition, it has been recently realized that this process also supports extracellular antigen processing for MHC class II presentation and cross-presentation on MHC class I molecules. Although the exact mechanisms for the regulation of these antigen processing pathways by autophagy are still unknown, recent studies, summarized in this review, suggest that they contribute to immune responses against infections and to maintain tolerance. Moreover, they are targeted by viruses for immune escape, and could maybe be harnessed for immunotherapy.

## Introduction

T cells detect infected or transformed cells via antigen presentation by major histocompatibility complex (MHC) molecules on the cell surface. Helper $CD4^+$ T cells and cytotoxic $CD8^+$ T cells are restricted by MHC class I and II molecules, respectively. These molecules display antigens in the form of peptides on the cell surface. These peptides are generated via distinct proteolytic processes and loaded in different cellular compartments on MHC class I and II molecules. Some antigen processing for peptide presentation by MHC molecules is assisted by autophagy.

$CD8^+$ T cells, which rapidly expand during immune responses and mediate cytotoxicity against infected and tumor cells, recognize octamer or nonamer peptides on MHC class I molecules [1]. These peptides are thought to originate primarily from protein degradation by proteasomes, large multicatalytic proteases in cytosol and nucleus [2]. These peptides are then imported into the endoplasmic reticulum (ER) via the transporter associated with antigen processing (TAP), and can be further trimmed on their N-terminus via ER aminopeptidases associated with antigen processing (ERAAPs) [3]. Peptides that fulfil the binding requirements for the expressed MHC class I molecules, so-called binding motifs, are then loaded into the peptide binding groove of these molecules in the MHC class I loading complex. Upon binding of high-affinity ligands, MHC class I molecules and their peptide cargo are then released from the ER to travel to the cell surface for immune surveillance by $CD8^+$ T cells. This model of antigen loading onto MHC class I molecules predicts that proteasome substrates are the main source of MHC class I ligands. Originally, it was assumed that MHC class I molecules would, therefore, primarily present cytosolic and nuclear antigens. However, it was noted that certain cell types, primarily professional antigen presenting cells like dendritic cells (DCs) are capable to display extracellular antigens on MHC class I molecules by antigen processing via cross-presentation [4-5]. During cross-presentation endocytosed antigen is thought to escape into the cytosol by so far poorly defined mechanisms. Whole proteins or preprocessed protein fragments might be delivered to the cytosol and at least for some antigens vesicular degradation by insulin regulated

aminopeptidase (IRAP) is required for efficient crosspresentation [6]. In the cytosol cross-presented antigens can access the proteasome and follow the MHC class I antigen processing and presentation pathway. The stage of endosomal maturation, from which cross-presented antigen is exported, is heavily debated, and early endosomes as well as late endosomes, including some that might even fuse with the ER, have been implicated [7-10]. Thus different antigen formulations might be cross-presented from different endosomes, but, as we will discuss later, antigen assisted in its exocytosis by macroautophagy might access one of these cross-presenting compartments very efficiently.

In contrast to MHC class I molecules, MHC class II molecules present longer peptides of heterogenic size (≥9 amino acids long) to $CD4^+$ T cells, which orchestrate humoral and cellular immune responses by virtue of their cytokine production. MHC class II ligands are primarily products of lysosomal proteolysis [11], and are loaded with these in late endosomal compartments, called MHC class II loading compartment (MIIC). MHC class II molecules reach these late endosomes with the help of a chaperone called invariant chain (Ii), which prevents premature peptide binding of MHC class II molecules in ER and Golgi apparatus, as well as guides MHC class II molecules via its cytosolic domain to MIICs. In these vesicles Ii is degraded by lysosomal proteolysis, and the remnant peptide occupying the peptide binding groove, class II-associated invariant chain peptide (CLIP), is expelled with the help of the chaperones H2-M or HLA-DM in mouse and man, respectively. These chaperones also ensure high affinity peptide ligand binding to MHC class II molecules. Thus, MHC class II presented antigens are in their majority proteins that can gain access to the MIIC. In the classical paradigm of MHC class II antigen processing, these were thought to be exclusively extracellular protein that reach the MIIC after endocytosis. As discussed later, it has now been realized that autophagy can permit cytoplasmic antigen to also access this pathway.

With respect to autophagic pathways that can be involved in antigen processing for MHC presentation, only macroautophagy and chaperone-mediated autophagy have so far been investigated, and we want to briefly introduce the processes and molecules that have been used by immunologists to elucidate a role for autophagy in antigen presentation to T

cells. During macroautophagy more than 30 autophagy-related gene (*atg*) products are involved in the generation and lysosome fusion of cytosolic double-membrane surrounded vesicles, called autophagosomes [12]. The sites of autophagosome generation are characterized by the assembly of type III phosphoinositide-3 (PI3) kinase complexes, incorporating Atg6/Beclin-1. Both rough ER and the outer nuclear leaflet have been identified as autophagosome formation sites [13-15], but these vesicles might also be generated at other places. Two ubiquitin-like systems are involved in autophagosome formation at these sites with Atg8 and Atg12 as the ubiquitin-like molecules at their center [16]. In one ubiquitin-like system Atg12 gets coupled to Atg5 with the assistance of the E1- and E2-like enzymes Atg7 and Atg10. The Atg12-Atg5 heterodimer then associates with Atg16L1 and coates the outer autophagosome membrane. It dissociates from the outer membrane, once the autophagosome is completed, and has been shown to direct the conjugation of the other ubiquitin-like molecule Atg8. Atg8 and its most studied mammalian homologue LC3 are coupled to the lipid phosphatydilethanolamine at the outer and inner autophagosomal membrane with the help of the E1- and E2-like enzymes Atg7 and Atg3. While it is recycled from the outer membrane upon autophagosome completion, it stays associated with the inner membrane and is degraded with the autophagosome cargo in lysosomes. Atg8/LC3 catalyses hemifusion of membranes and might therefore support the elongation of the autophagosome membrane [17]. In addition, it is used to recruit autophagic cargo [18-20]. In addition to its role in autophagosome formation, Atg6/Beclin-1 containing PI3 kinase complexes are then once more involved in macroautophagy at the stage of autophagosome fusion with the lysosome [21-24]. In addition to macroautophagy, chaperone-mediated autophagy also imports cytosolic proteins into lysosomes for degradation [25]. Its substrates carry a KFERQ-like signal sequence, which targets them for direct transport across the lysosomal membrane. This transport is assisted by cytosolic and vesicular chaperones of the heat-shock protein family and the transmembrane protein LAMP2A. Thus both macroautophagy and chaperone-mediated autophagy deliver cytoplasmic constituents to lysosomes for degradation. Since T cells monitor the proteolytic waste of cells for signs of

infection and transformation, it is maybe not surprising that they also survey substrates of autophagy. The evidence for this will be discussed below.

## Antigen processing for MHC class II presentation via autophagy

### *Endogenous pathway*

Different studies have now demonstrated that autophagosomes can deliver cytosolic and nuclear proteins to MHC class II loading compartments (Figure 1), and this phenomenon occurs in different cell types: in epithelial cells, in B cells, but also in dendritic cells, the most professional antigen presenting cells. Morphological analysis of MHC class II compartment have revealed that betwheen 30 and 50 % of MHC class II compartment co-stain with the autophagosome marker Atg8/LC3 [26]. In parallel, using different model antigens, for the generation of which proteins were coupled to Atg8/LC3, our group demonstrated that targeting an antigen to macroautophagy significantly enhances its processing and presentation on MHC class II molecules [26] and (Gannage et al., unpublished observation). In this section we discuss different examples of intracellular antigens from self, viral and tumor origin that have been shown to be processed for MHC class II presentation via macroautophagy.

Evidence that self antigens can be delivered to MHC class II loading compartment via macroautophagy came first from the analysis of MHC class II bound epitopes. In human B lymphoblastoid cell lines, mass spectrometrical analysis of the MHC class II ligandome showed that 20 to 30% of self class II epitopes are derived from cytosolic and nuclear proteins. A significant change in the MHC presented amount of these peptides occurred upon starvation induced macroautophagy [27]. This self-antigen presentation via macroautophagy might be involved in autoimmune diseases. Along these lines, the group of Ludger Klein has implicated macroautophagy in the generation of central tolerance by MHC class II antigen presentation of self antigens on thymic epithelial cells (TECs). Using a model of transplantation of $Atg5^{-/-}$ thymi into wild type or TCR transgenic mice, Klein et al. demonstrated that thymic macroautophagy shapes the repertoire of $CD4^{+}$ T cells [28]. Macroautophagy in cortical TECs seemed to be required for positive selection of certain T cell receptor specificities, while in medullary TECs it was required for negative selection.

Without macroautophagy dependent negative selection, immunopathology was observed in several tissues including the gut, and the symptoms could be transferred by T cells. Similarly, Kasai and colleagues demonstrated by immunofluorescence analysis the colocalization of Atg8/LC3 with MHC class II loading compartment in TECs, both in vitro (in cTEC and mTEC cell lines) and in vivo (in thymic cryosection) [29]. In addition, Western blot analysis of H2-DM vesicles isolated from TECs lines detected the lipidated form of Atg8/LC3 (LC3-II), suggesting fusion of autophagosomes with these MHC class II loading compartments. It is tempting to speculate that macroautophagy allows intracellular antigens, including peripheral tissue derived antigen that are transcribed due to the expression of the transcription factor autoimmune regulator element (AIRE), to be presented onto MHC class II molecules for central tolerance induction by TECs. However, this self-antigen presentation might also result in autoimmunity, and Janice Blum's group has indeed shown that the autoantigens GAD65 and SMA can be better presented to $CD4^+$ T cells when chaperone-mediated autophagy is enhanced [30]. These data suggest that both macroautophagy and chaperone-mediated autophagy transport self-proteins for MHC class II presentation to $CD4^+$ T cells during tolerance induction and possibly also autoimmune disease.

Apart from self-antigens, macroautophagy can also deliver pathogen-derived antigens to MHC class II loading compartments. This is exemplified by viral antigens in Epstein Barr virus (EBV) infected B cells. The pathway has been implicated in the degradation of two latent EBV antigens, EBV nuclear antigen 1 (EBNA1) and latent membrane protein 1 (LMP1). LMP1 which is considered the major oncogene of EBV, induces macroautophagy [31] and accumulates upon silencing of the two essentiel macroautophagy gene products Atg6/Beclin 1 and Atg7. Therefore, LMP1 seems to regulate its own clearance by macroautophagy, but further studies will have to be performed to demonstrate if this results in $CD4^+$ T cell epitope generation from LMP1. For the second latent EBV antigen that is at least partially turned over by macroautophagy, EBNA1**,** antigen processing for MHC class II presentation via macroautophagy has been demonstrated [32-33]. Leung et al. identified 2 epitopes from EBNA1 that are processed via endogeneous pathways for MHC class II

presentation by EBV infected B cells. In line with previously published results from our group [32], one of these epitopes is delivered to MHC class II loading compartments via macroautophagy. Interestingly, preventing nuclear import of EBNA1 by mutating its nuclear localization sequence, strongly enhanced the presentation of both this and the other $CD4^+$ T cell epitopes. Both of them were now delivered to MHC class II loading compartment via macroautophagy. Thus, cytosolic relocation of EBNA1 resulted in broadening the range of $CD4^+$ T cell epitopes, displayed on MHC class II molecules after macroautophagy. How the relocation of an antigen from the nucleus to the cytosol can enhance its endogeneous delivery to MHC class II compartment by macroautophagy remains unclear. In contrast to this finding, the study by Riedel et al. [34], suggested that for endogenous MHC class II presentation of the cytosolic bacterial antigen neomycin phosphotransferase II (NeoR) via macroautophagy, the relocation of the antigen to the nucleus does not affect its antigen recognition. The differences in these two studies might be related to the nature and expression level of the antigen itself, allowing them to form cytosolic malfolded protein pools to different degrees. In another viral infection, Herpes simplex virus type 1 (HSV-1) induces macroautophagy, but this response is antagonized by the HSV-1 neurovirulence gene product, ICP34.5, which interacts with the essential autophagy protein Atg6/Beclin-1. Interestingly, a mutant virus unable to bind to the Atg6/Beclin-1 protein induces a significantly stronger $CD4^+$ T cell response in infected mice [35]. This might imply an essential role of macroautophagy mediated MHC class II presentation during HSV-1 infection. Indeed, Akiko Iwasaki and colleagues have recently demonstrated that HSV infection of mice, lacking macroautophagy in DCs, was compromised in raising virus specific $CD4^+$ T cell responses [36]. Finally, in macrophages and DCs infected with mycobacterium tuberculosis (Mtb), induction of macroautophagy by rapamycin, IFN-$\gamma$ or starvation significantly enhanced the processing of immunodominant $CD4^+$ T cell epitopes from the mycobacterial antigen Ag85B [37]. This mechanism is an active process, occurring only in live Mtb infection, and is specifically inhibited by RNA silencing of Atg6/Beclin-1. In addition, vaccination of mice with rapamycin pretreated Mtb infected DCs significantly improved the efficiency of T cell responses prior to

Mtb challenge. This work described for the first time how the manipulation of macroautophagy could improve a vaccine strategy. Thus, MHC class II presentation of individual viral antigens via macroautophagy, a role for this pathway in vivo and even immune evasion by viral inhibition of macroautophagy has been demonstrated.

A third group of antigens that might fall prey to macroautophagy, are tumor antigens. The implications of macroautophagy during tumorigenesis are still unclear. Among others, macroautophagy has been shown to promote cell survival of cancer cells, but also in parallel to function as a tumor suppressor pathway in some models. Nevertheless, the pathway is active in cancer cells and might generate tumor-derived $CD4^+$ T cell epitopes. As an example, the protein encoded by the Mucin gene 1 (MUC1) has been described to be processed by macroautophagy after expression in DCs [38]. In MUC1 transfected DCs the processing of MUC1 for MHC class II presentation was found to be dependent on macroautophagy. This was documented by significant inhibition of MUC1 specific $CD4^+$ T cell proliferation during coculture with antigen expressing DCs in the presence of PI3 kinase inhibitors (3-methyladenine or wortmannin), which compromise autophagosome generation. In contrast, MUC1 specific $CD8^+$ T cell responses were unaffected by this pathway. Thus, self-, pathogen-derived and tumor-antigens have now been demonstrated to follow macroautophagy for MHC class II presentation to $CD4^+$ T cells. This pathway of immune surveillance should be harnessed during vaccination and for the development of novel immunotherapies.

***Exogeneous pathway***

In addition to its role in MHC class II presentation of cytosolic antigens, macroautophagy has recently been shown to participate in the processing of exogeneous antigens and their delivery to MHC class II compartment (Figure 1). Evidence for this pathway has been gathered primarily in macrophages and DCs. Indeed, three recent studies have established a role for macroautophagy in the processing of phagocytosed antigens for MHC class II presentation by DCs. Particularly, enhanced antigen processing after DC activation by

pathogen associated molecular patterns (PAMPs), as recognized by Toll-like receptors (TLRs) and NOD-like receptors (NLRs), was supported by macroautophagy.

The first study by the group of Akiko Iwasaki highlighted the importance of macroautophagy in DCs for $CD4^+$ T cell priming in vivo [36]. Interestingly, during herpes simplex virus (HSV) infection, macroautophagy seems to be important not only for the processing of cytosolic endogeneous antigens and their delivery to MHC class II compartment, but also for the processing of exogenous phagocytosed antigens. Using chimeric mice reconstituted with a hematopoietic system deficient for Atg5, the authors could demonstrate a defect in $CD4^+$ T cell responses in vivo upon HSV infection. In addition, in these chimeric mice, adoptively transferred ovalbumin (OVA)-specific $CD4^+$ T cells showed an impairment of proliferation after infection with OVA-expressing Listeria monocytogenes or OVA expressing HSV. A role of macroautophagy for MHC class II antigen processing and presentation in vivo in professional antigen presenting cells was further supported by mice with a conditional deletion of Atg5 in their DCs. Indeed in these mice HSV infection failed to prime $CD4^+$ T cell responses in vivo, and the mice succumbed to a more severe disease compared to wild type mice, and developed a higher clinical score upon infection. In the same study, the authors ruled out a general defect in macroautophagy deficient DCs, by demonstrating that they had no defect in their migration capability, in their phenotypic maturation (CD86, CD40 and MHC class II expression), in their cytokines production and were not impaired in endocytosis or phagocytosis compare to wild type DCs. Instead, the defect in extracellular antigen presentation on MHC class II molecules was rather due to an impairment of processing of phagocytosed antigens that trigger TLR stimulation. Using lipopolysaccharide (LPS) coated OVA beads, an impairment of phagosome to lysosome fusion was observed in $Atg5^{-/-}$ DCs, resulting in a reduction of the processing of phagocytosed antigen for MHC class II presentation. This phenotype was the consequence of a defect in the delivery of lysosomal proteases to phagosomes, as well as a delay of fusion of phagosomes with lysosomes. The precise mechanism of how the macroautophagy machinery is recruited to phagosomes and might enhance fusion with lysosomes remains

unclear, but these findings are in line with previous studies, documenting enhanced antigen presentation of TLR ligand coated antigen and Atg supported fusion of phagosomes containing such antigen with lysosomes [39-40]. In one of these studies, the authors described that TLR engagement induces “LC3-associated phagocytosis” (LAP). Upon TLR2 stimulation a recruitment of Atg8/LC3 to the phagosomal membrane was observed, resulting in an enhanced maturation of the phagosome. These studies suggest that macroautophagy facilitates endocytosed antigen delivery to lysosomes, and MHC class II presentation of this antigen.

Along the same lines, Alison Simmons and colleagues demonstrated that upon NOD2 (nucleotide-binding oligomerization domain-containing-2) activation, macroautophagy is upregulated and involved in MHC class II processing of phagocytosed antigens [41]. This study provided for the first time evidence that a defect in macroautophagy mediated MHC class II antigen presentation could result in defective $CD4^+$ T cell responses, which might then not be able to control the gut commensals in Crohn disease (CD). This defect in macroautophagy mediated MHC class II antigen presentation could be caused by mutations in both the Atg16L1 and NOD2 gene, which had been identified as major risk factors for the occurrence of the disease. The authors demonstrated that a bacterial peptidoglycan muramyl dipeptide (MDP) induces macroautophagy upon NOD2 activation in human DCs. MHC class II expression on the cell surface of MDP activated DCs was upregulated and this up-regulation was dependent on macroautophagy. In parallel a colocalization of Atg8/LC3 with MHC class II loading compartments was observed. Moreover, DC-mediated priming of $CD4^+$ T lymphocytes after Salmonella enterica infection was dependent on NOD2 and Atg16L1, because $CD4^+$ T cell proliferation was compromised by siRNA mediated silencing of these two genes. The second part of this study analyzed the function of DCs from CD patients, carrying either the NOD (3020insC) or the Atg16L1 (T300A) mutation, which are associated with the familial form of the disease. In both cases, the cells demonstrated a defect in macroautophagy up-regulation upon MDP stimulation or Salmonella enterica infection. As a consequence MHC class II upregulation and $CD4^+$ T cell proliferation were

compromised. Confirming these findings, the group of Dana Philpott could demonstrate that NOD1 and 2 stimulation up-regulated macroautophagy and led to autophagosome recruitment to the plasma membrane [42]. This regulation was independent of the classical pathway of NLR activation, involving usually the RIP2 adaptor and NF-κB transcription factor. These data suggested that also NLR-ligand stimulated MHC class II up-regulation and antigen presentation requires macroautophagy.

Finally, a third recent study describes a new mechanism of immune escape of HIV-1 virus in DCs [43]. In this work, HIV-1 was shown to inhibit macroautophagy initiation by activating the mTor pathway. This inhibition resulted in an impairment of TLR4 and TLR8 activation by viral replication intermediates and of antigen presentation to $CD4^+$ T cells. Indeed in an in vitro essay of DC capture of inactivated HIV virus, macroautophagy was shown to be important for MHC class II antigen processing of a HIV gag-derived $CD4^+$ T cell epitope. TNF-α secretion of a HIV gag specific $CD4^+$ T cell clone, was significantly reduced upon stimulation with macroautophagy deficient DCs, pulsed with inactivated HIV virus. In parallel, $CD8^+$ T cell activation was not compromised by macroautophagy inhibition. This study suggests that efficient HIV antigen processing for MHC class II presentation by DCs requires macroautophagy.

**Macroautophagy in antigen processing for MHC class I presentation**

In contrast to a quite robust role for macroautophagy in MHC class II antigen presentation, much less is known about how this pathway influences antigen processing for MHC class I presentation. Indeed in several studies MHC class I restricted antigen recognition was actually used as a control and no effect of macroautophagy inhibition on $CD8^+$ T cell recognition was observed [26,36,43]. However, under certain circumstances macroautophagy might enhance antigen presentation on MHC class I molecules to $CD8^+$ T cells, and we will summarize the so far sparse evidence for this here.

The only study so far that argues for a role of macroautophagy in endogenous antigen processing for MHC class I presentation by the group of Michel Desjardins suggested that late during HSV infection macroautophagy supports MHC class I presentation of viral antigens [15]. This in vitro finding was restricted to an immunodominant $CD8^+$ T cell epitope of the glycoprotein B of the virus, and to its processing during the late stage of the infection. Interestingly early after infection (6-8h), the presentation of this epitope followed the classical MHC class I pathway, but later on (8-12h), macroautophagy inhibition compromised presentation to $CD8^+$ T cells, even so processing of this antigen was still dependent on components of the classical MHC class I antigen processing pathway like the proteasome and TAP. In infected macrophages, the authors demonstrated that silencing the essential autophagy gene *atg5* resulted in a decrease of HSV gB specific $CD8^+$ T cell recognition. Electron microscopy analysis revealed that the formation of autophagosomes after HSV-1 infection involved the outer nuclear membrane. However, it was not clarified by which mechanism autophagosome cargo can escape to the cytosol to be further processed by proteasomes. Irrespective of the mechanism, this study suggests that macroautophagy might intersect with the cross-presentation pathway of MHC class I antigen processing.

A similar intersection with endosomes capable of cross-presentation was proposed for macroautophagy substrates of antigen donor cells (Figure 1). The first report showing a direct involvement of macroautophagy in crosspresentation was by Li and colleagues [44]. In this study 293T cells expressing the model antigen OVA in vitro, or melanoma cells expressing the gp100 melanoma antigen in vivo, were used as donor cells to access crosspresentation. In both cases siRNA mediated silencing of macroautophagy in antigen donor cells resulted in a significant reduction of $CD8^+$ T cell activation after cross-presentation by DCs. Interestingly, purified autophagosomes from antigen donor cells were also efficiently cross-presented to $CD8^+$ T cells, probably acting like exosomes. Therefore, antigen wrapped in macroautophagic membranes might be more efficiently taken up by antigen presenting cells and cross-presented on MHC class I molecules.

In a second report along these lines by the group of Matthew Albert [45], evidence was provided that efficient cross-presentation of viral antigens required macroautophagy in antigen donor cells. In this study two cellular sources were used to provide antigen for cross-presentation experiments, wild type mouse embryonic fibroblasts (MEFs) and Bax/Bak$^{-/-}$ MEFs. While wild-type MEFs can undergo caspase-dependent apoptosis, Bax/Bak$^{-/-}$MEFs are unable to perform this type of cell death, but up-regulate macroautophagy under the applied treatment conditions. Both cell types were infected with influenza A virus, than treated with proapoptotic agents and adoptively transferred in vivo for priming experiment. Mice immunized with Bax/Bak$^{-/-}$ MEFs showed a significantly higher CD8$^{+}$ T cell response specific to both $HA_{518-526}$ and $NP_{366-374}$ immunodominant CD8$^{+}$ T cell epitopes, compared to mice immunized with wild-type MEFS. These findings again suggest that macroautophagy might package antigen efficiently for cross-presentation by DCs. How macroautophagic substrates, however, leave antigen donor cells for cross-presentation, remains to be determined.

**Viral evasion from macroautophagy**

The important role of macroautophagy during immune responses is further underscored by its regulation during viral infections. Three in vivo systems have linked macroautophagy to immune control of viral infections. These included with Herpes simplex virus (HSV) one DNA and with Sindbis virus and vesicular stomatitis virus (VSV) two RNA virus. Neurovirulence of both HSV and Sindbis virus was attenuated when macroautophagy was elevated either by mutation of the macroautophagy inhibiting ICP34.5 HSV gene product [46], or by overexpression of Atg6/Beclin-1, driving macroautophagy, in Sindbis virus infected mice [47]. Furthermore, Sindbis virus infection of the CNS is augmented by macroautophagy inhibition via either recombinant viruses encoding a dominant negative Atg5 protein, recombinant viruses deleting floxed Atg5 upon infection via cre-recombinase expression or wild-type virus infection of mice with conditional Atg5 deletion in neurons [48]. In addition, VSV infection induced macroautophagy in Drosophila flies, and restricted VSV infection in this model organism [49]. These studies did not implicate antigen processing for MHC presentation via macroautophagy in the protection from virus infection. However, during HSV infection, macroautophagy in myeloid DCs was found to be required for efficient priming of protective CD4+ T cell responses [36]. These studies implicate that macroautophagy restricts viral replication in vivo.

Another indication that macroautophagy significantly limits viral replication is that successful viral pathogens have developed immune escape mechanisms to target this pathway. In this respect, two checkpoints of macroautophagy are inhibited by viruses. These are autophagosome formation and autophagosome fusion with lysosomes. Interestingly, DNA viruses seem to primarily compromise autophagosome formation, while RNA viruses block autophagic cargo degradation in lysosomes. With respect to the inhibition of autophagosome formation, herpesviruses have proven a rich source of macroautophagy inhibiting proteins. Within the $\alpha$-herpesviruses, HSV encodes the late infected cell protein (ICP) 34.5, which blocks autophagosome formation [50-51]. ICP34.5 inhibits autophagosome generation by binding to Atg6/Beclin-1 [46]. Interestingly, HSV with a mutant ICP34.5 protein,

lacking the Atg6/Beclin-1 interacting domain, shows increased neurovirulence after CNS injection in mice [46]. Within the β-herpesviruses, human cytomegalovirus (HCMV) also inhibits macroautophagy [52]. However, the molecular mechanism of this regulation is unknown so far. Finally, the group of γ-herpesviruses contains several members that compromise autophagosome formation, namely Kaposi sarcoma associated herpesvirus (KSHV) and murine γ-herpesvirus (MHV-68). KSHV encodes a B-cell lymphoma 2 protein (Bcl-2) homologue, encoded by ORF-16 of the virus, which also interacts with Atg6/Beclin-1 and inhibits autophagsome formation [53]. In addition, the same virus compromises autophagosome formation also via its viral FLICE inhibitory protein (v-FLIP) protein, encoded by ORF71/K13 [54]. KSHV v-FLIP impairs Atg3, the E2-like enzyme of Atg8/LC3 lipidation. This mechanism prevents Atg8/LC3 dependent autophagosome formation. Therefore, KSHV expresses with v-FLIP a macroautophagy inhibitor during latent, and with v-Bcl-2 one during lytic infection. The closest rodent virus to KSHV, MHV-68 also expresses a Bcl-2 homologue, M11, which interacts with Atg6/Beclin-1 to inhibit autophagosome formation [55]. Deletion of the macroautophagy blocking, but not the apoptosis compromising domain, impairs the establishment of chronic infection by this virus [56]. Collectively, these data suggest that herpesviruses inhibit autophagosome formation in order to inhibit restriction of viral infection and viral antigen presentation.

In addition to autophagosome generation as a first checkpoint of macroautophagy, degradation of these vesicles via fusion with lysosomes is targeted by RNA viruses. Already early electron microscopy studies of poliovirus infected cells reported the accumulation of double-membrane engulfed vesicles [57]. This stabilization of autophagosomes could be induced by the viral proteins 2BC and 3A [58], even so the molecular mechanism underlying this presumed block in autophagosome degradation remains unclear. Formation of these poliovirus stabilized vesicles is dependent on some Atg proteins, because siRNA mediated silencing of Atg8/LC3 and Atg12 inhibits their generation, and they seem to represent, therefore, a subtype of autophagosomes [58]. The stabilized membranes support viral replication and are thought to function as a scaffold for viral replication. In addition, inhibition

of autophagosome degradation might enhance virus particle exocytosis [58]. Furthermore, the stabilization of autophagosome membranes for viral replication has been proposed for other RNA viruses, like the flaviviruses hepatitic C virus (HCV) and dengue virus [59-63]. Moreover, even RNA viruses that do not use autophagosomal membranes for their replication, seem to benefit from inhibition of autophagosome degradation for their release of infectious viruses from infected cells [64]. Along these lines the human immunodeficiency virus (HIV) was found to block autophagosome maturation. Particularly its Nef protein interacts with Atg6/Beclin-1 blocking degradation of macroautophagic cargo, including HIV particles. These, therefore, are exocytosed at increased rates. This benefit for HIV in blocking autophagosome degradation was only observed in macrophages, but not T cells. Similarly, a slight benefit of macroautophagy regulation for the replication of the segmented RNA virus influenza virus was observed only in canine kidney epithelial cells, but not in human lung epithelia or mouse embryonic fibroblasts [65-66]. Irrespective of a benefit for viral replication, influenza infection leads to autophagosome accumulation in a broad range of cell types [66]. The virus achieves this by blocking autophagosome fusion with lysosomes via its matrix protein 2 (MP2). This macroautophagy block enhances apoptotic cell death of infected cells and might regulate immunogenic viral protein release. Taken together these studies suggest that viruses highjack macroautophagy to prevent their degradation and viral antigen presentation, or even use autophagic membranes for their own replication and exit from infected cells. Depending on the need of the virus they block autophagosome generation or degradation with DNA viruses targeting preferentially the first, and RNA viruses the second check-point of macroautophagy, respectively.

## Conclusions

Macroautophagy supports adaptive immune responses by facilitating antigen processing for MHC presentation to T cells. Since autophagosomes direct cytoplasmic content for lysosomal degradation and MHC class II molecules present lysosomal products to $CD4^+$ T cells, most studies suggest a role for macroautophagy in antigen presentation to these helper T cells. However, some studies also describe that macroautophagy might even assist MHC class I antigen presentation to $CD8^+$ T cells. While intracellular antigen presentation by MHC class II molecules seems to benefit from macroautophagic substrate delivery to MHC class II loading compartments, the mechanisms by which extracellular antigen delivery to these MIICs is supported by macroautophagy, by which autophagosome content can again escape to the cytosol for classical MHC class I antigen presentation, and by which antigen can be efficiently packaged by macroautophagy for cross-presentation remain unclear. The evidence that viruses, however, target this pathway for their immune escape suggests that it might be important for immune control. Thus, macroautophagy should be harnessed to enhance immunotherapies and assist antigen processing during vaccinations.

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**Figure legend**

**Figure 1: Macroautophagy assists in antigen processing for MHC class I and II presentation.** Cytosolic antigen can be directly transported to MHC class II loading compartments (MIIC) via autophagosomes (lower left). In addition, it might escape lysosomal degradation and get exocytosed from multivescular bodies (MVB) for uptake by bystander cells (right and upper left). The endocytosed antigen might get loaded onto MHC class II molecules or escape to the cytosol for proteasomal degradation and presentation on MHC class I molecules.

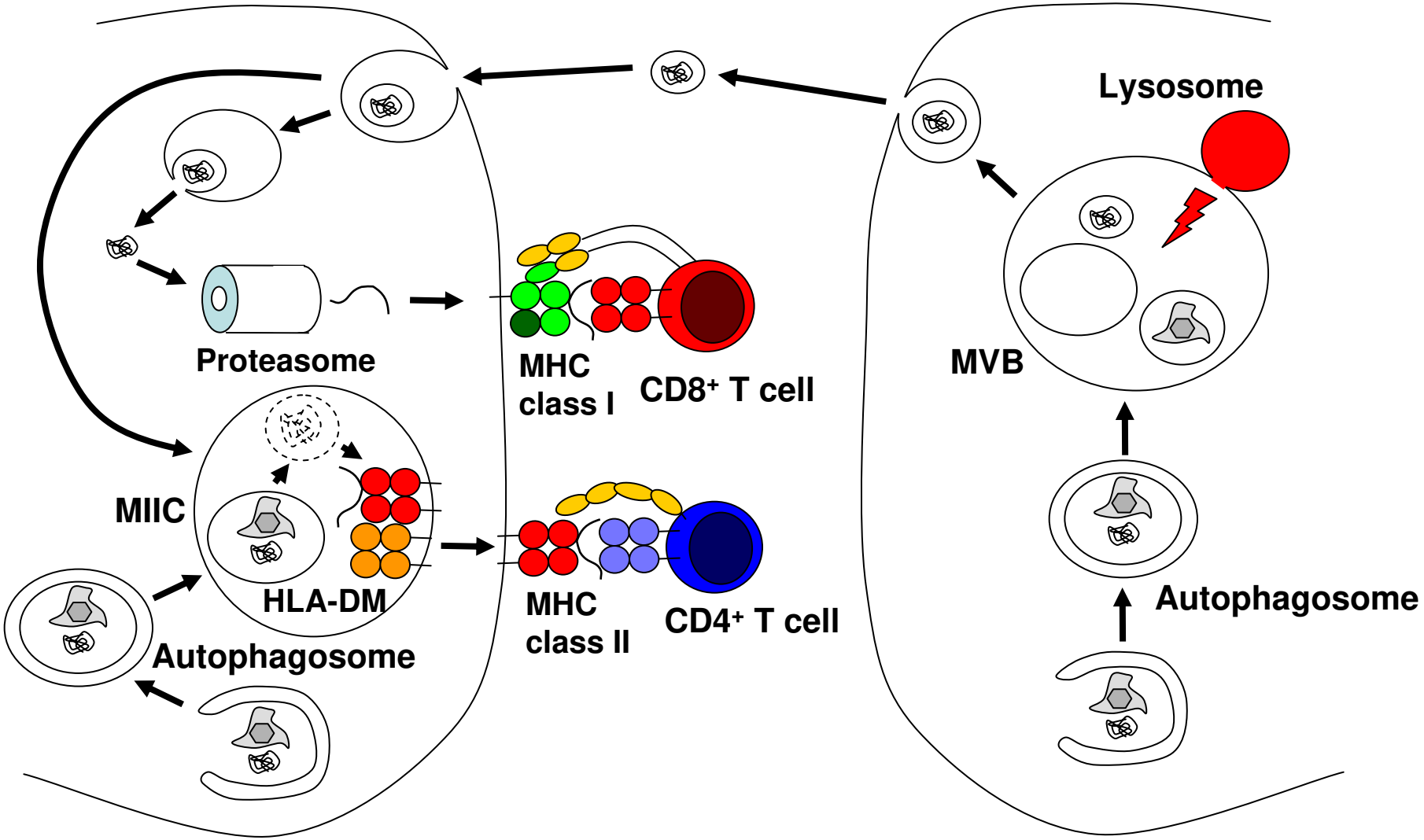
Lysosome
MVB
Proteasome
MHC
class I
CD8+ T cell
MIIC
HLA-DM
MHC
class II
CD4+ T cell
Autophagosome
Autophagosome

Figure 1